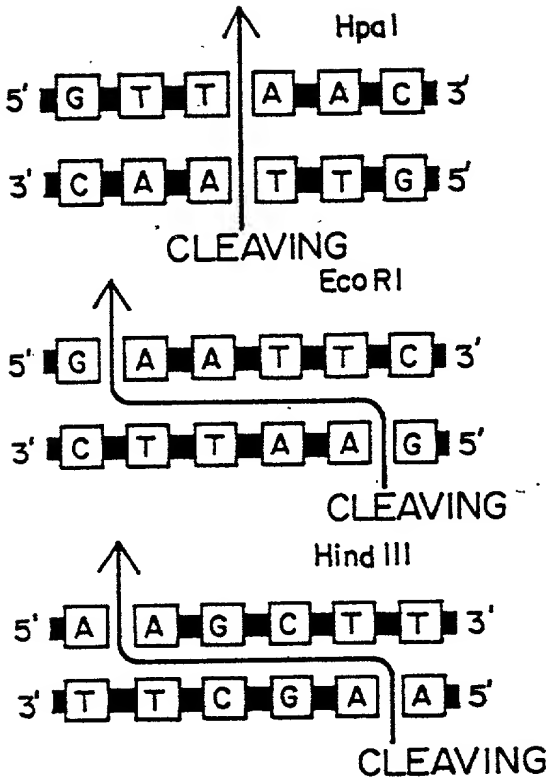
Hpa I
5' G T T A A C 3'
3' C A A T T G 5'
CLEAVING
Eco RI
5' G A A T T C 3'
3' C T T A A G 5'
CLEAVING
Hind III
5' A A G C T T 3'
3' T T C G A A 5'
CLEAVING

FIG. 1

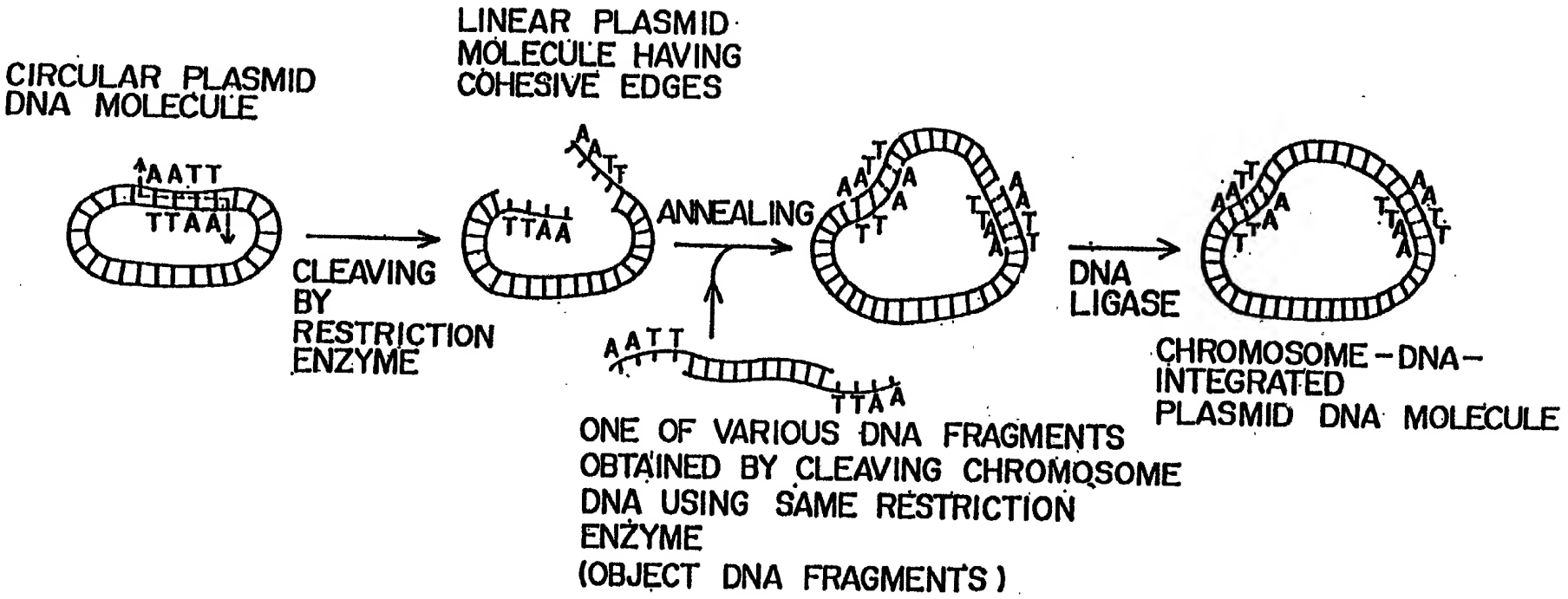
CIRCULAR PLASMID DNA MOLECULE
LINEAR PLASMID MOLECULE HAVING COHESIVE EDGES
AATT
TTAA
CLEAVING BY RESTRICTION ENZYME
ANNEALING
DNA LIGASE
CHROMOSOME-DNA-INTEGRATED PLASMID DNA MOLECULE
ONE OF VARIOUS DNA FRAGMENTS OBTAINED BY CLEAVING CHROMOSOME DNA USING SAME RESTRICTION ENZYME
(OBJECT DNA FRAGMENTS)

FIG. 2

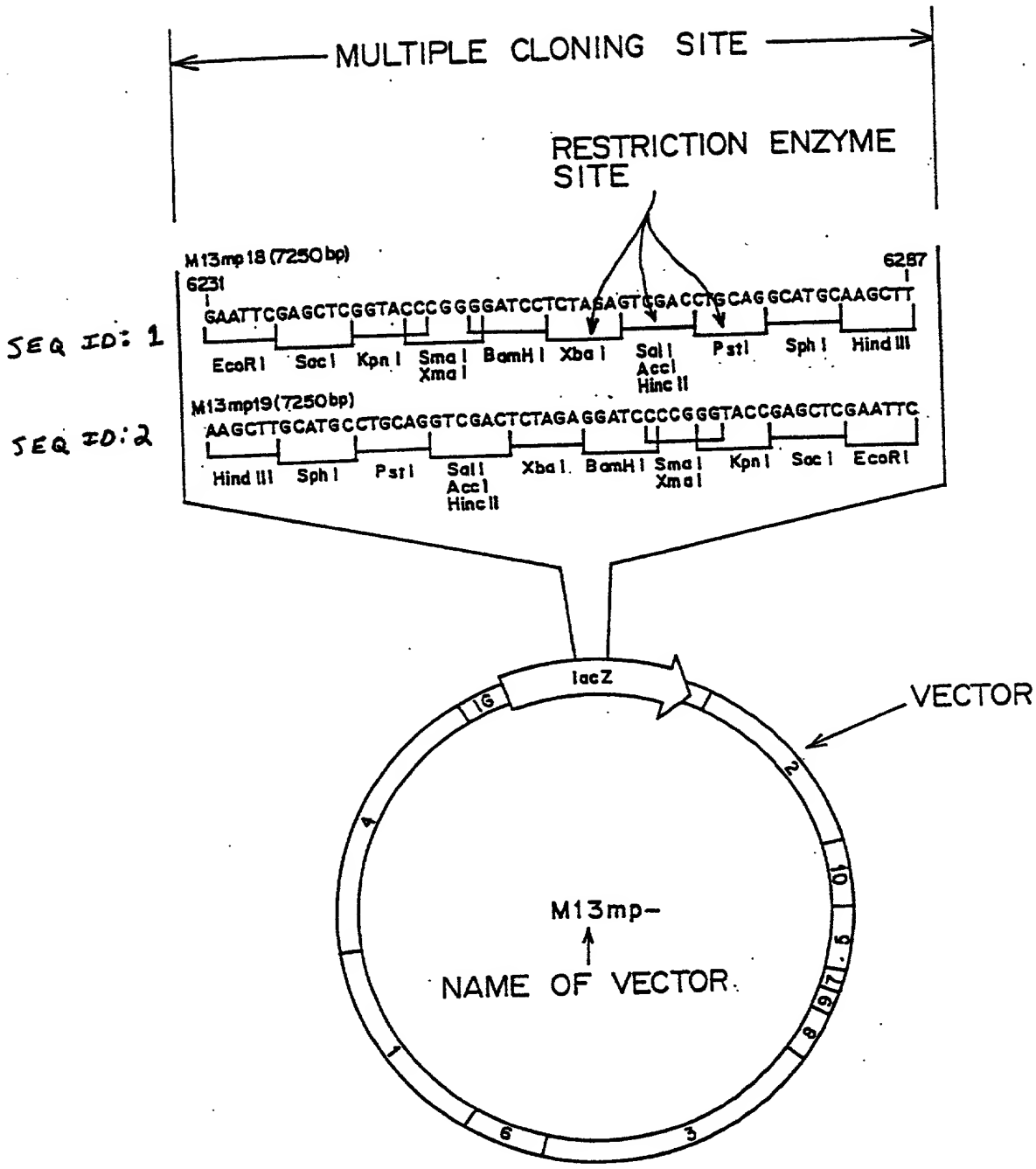
MULTIPLE CLONING SITE
RESTRICTION ENZYME SITE
M13mp18 (7250bp)
6231
6287
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT
EcoR I
Sac I
Kpn I
Sma I
Xma I
BamH I
Xba I
Sal I
Acc I
Hinc II
Pst I
Sph I
Hind III
SEQ ID: 1
M13mp19(7250bp)
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC
Hind III
Sph I
Pst I
Sal I
Acc I
Hinc II
Xba I
BamH I
Sma I
Xma I
Kpn I
Sac I
EcoR I
SEQ ID: 2
lacZ
VECTOR
M13mp-
NAME OF VECTOR

FIG. 3

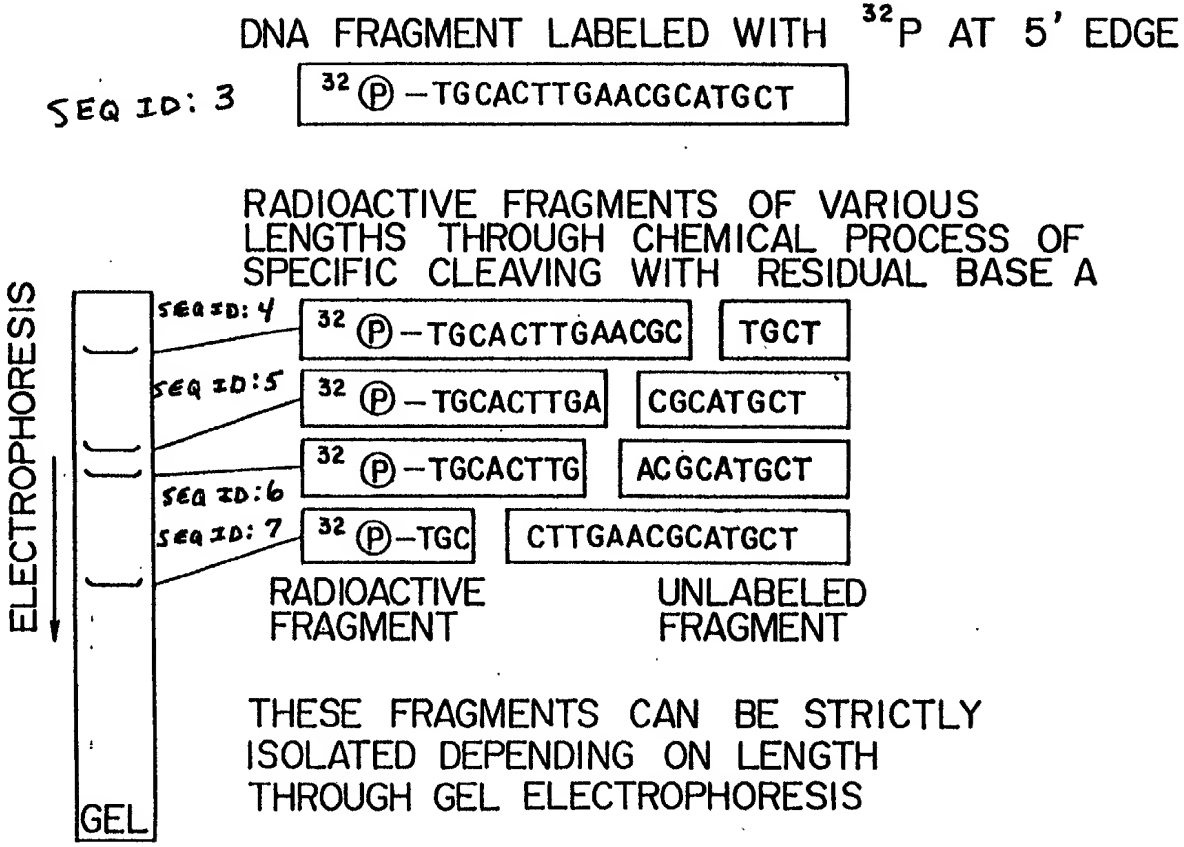
DNA FRAGMENT LABELED WITH 32P AT 5' EDGE
SEQ ID: 3
32Ⓟ–TGCACTTGAACGCATGCT
RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A
ELECTROPHORESIS
SEQ ID: 4
32Ⓟ–TGCACTTGAACGC
TGCT
SEQ ID: 5
32Ⓟ–TGCACTTGA
CGCATGCT
SEQ ID: 6
32Ⓟ–TGCACTTG
ACGCATGCT
SEQ ID: 7
32Ⓟ–TGC
CTTGAACGCATGCT
RADIOACTIVE FRAGMENT
UNLABELED FRAGMENT
GEL
THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4

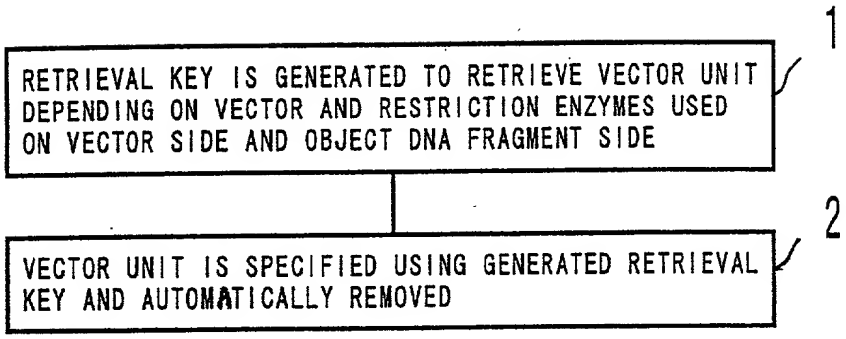
RETRIEVAL KEY IS GENERATED TO RETRIEVE VECTOR UNIT DEPENDING ON VECTOR AND RESTRICTION ENZYMES USED ON VECTOR SIDE AND OBJECT DNA FRAGMENT SIDE
1
VECTOR UNIT IS SPECIFIED USING GENERATED RETRIEVAL KEY AND AUTOMATICALLY REMOVED
2

FIG. 5

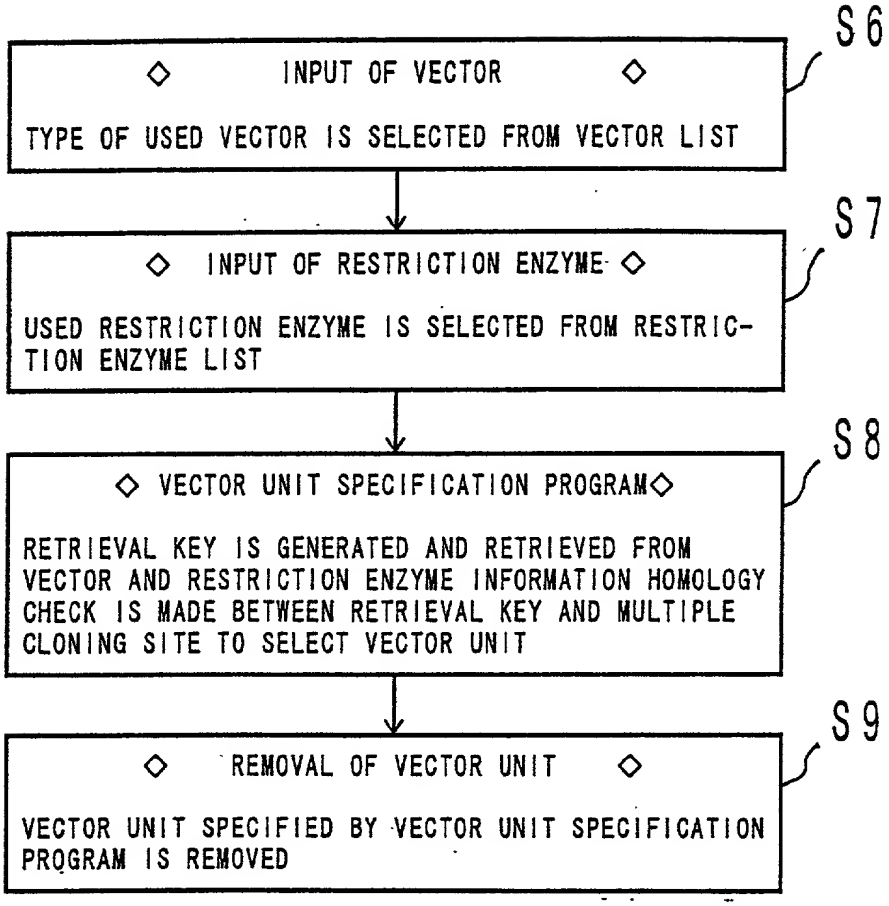

FIG. 6

EP 2 345 973 A1

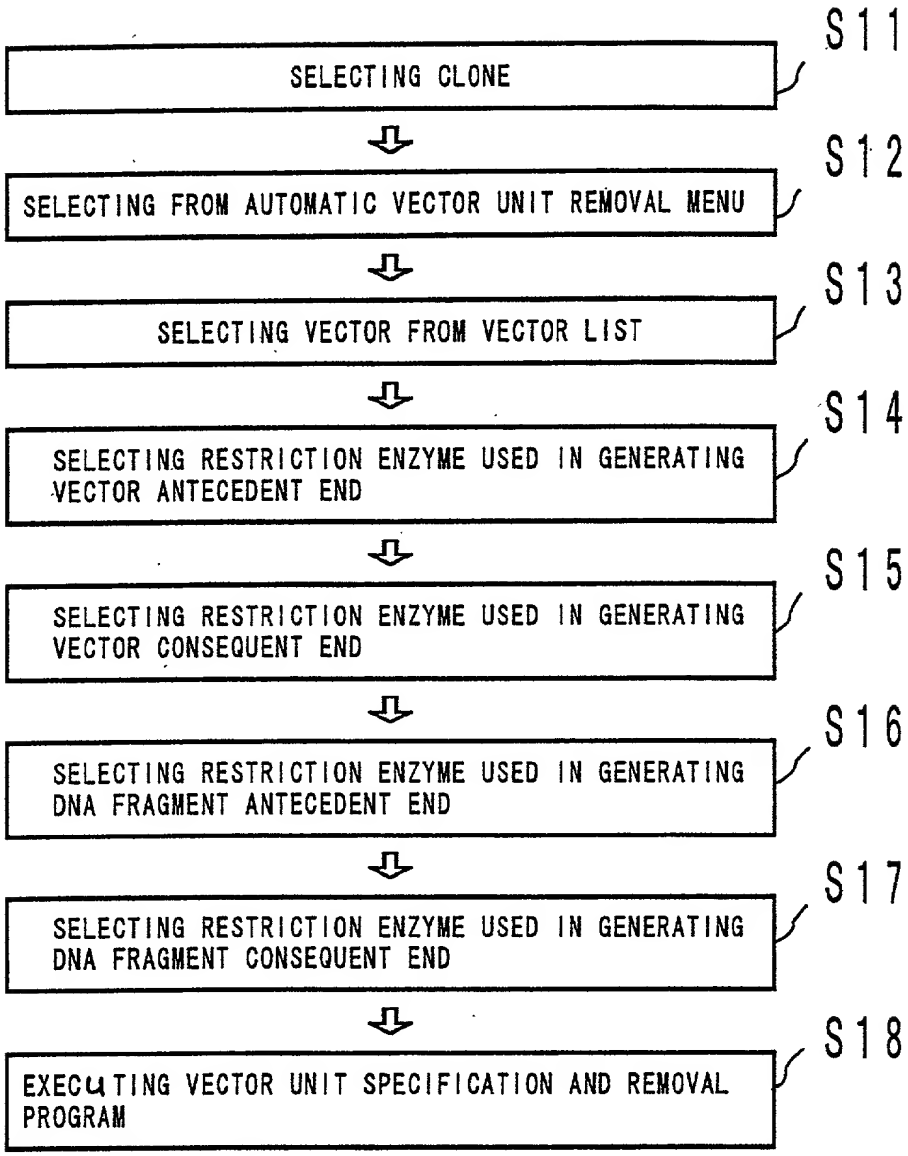
S11
SELECTING CLONE
S12
SELECTING FROM AUTOMATIC VECTOR UNIT REMOVAL MENU
S13
SELECTING VECTOR FROM VECTOR LIST
S14
SELECTING RESTRICTION ENZYME USED IN GENERATING VECTOR ANTECEDENT END
S15
SELECTING RESTRICTION ENZYME USED IN GENERATING VECTOR CONSEQUENT END
S16
SELECTING RESTRICTION ENZYME USED IN GENERATING DNA FRAGMENT ANTECEDENT END
S17
SELECTING RESTRICTION ENZYME USED IN GENERATING DNA FRAGMENT CONSEQUENT END
S18
EXECUTING VECTOR UNIT SPECIFICATION AND REMOVAL PROGRAM

FIG. 7

M13MP18
M13MP19
PBR322
PSL1180
PSL1190
PT7T318U
PT7T319U
PTZ18R
PTZ19R
PUC18
PUC19, ETC.

FIG. 8

0976565.0552502

VECTOR DB FORMAT

```
>ID
PUC18
>SEQ ID: 8
TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGA
GCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCC
ATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAA
GCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCT
GTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAAT
GAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
GCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
TTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC
GGGAAGCGTGGCGCTTTCTCAAAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTG
TGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC
TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG
TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGA
AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGAT
TTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCA
TCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTC
CTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCA
ACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAA
GTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGC
TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTC
AATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTC
ACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAAT
ACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTA
TTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATC
ATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC
>MULTI
399..450
```

FIG. 9

FOh250"69258260

(* INDICATES MULTIPLE CLONING SITE)

****

SEQ ID: 9 GTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT

SEQ ID: 10 AAGCTT⇨HIND III

SEQ ID: 11 GCATGC⇨SPH I

SEQ ID: 12 CTGCAG⇨PST I

SEQ ID: 13 GTCGAC⇨SAL I, ACC I, HINC II

SEQ ID: 14 TCTAGA⇨XBA I

SEQ ID: 15 GGATCC⇨BAMH I

SEQ ID: 16 CCCGGG ⇨SMA I, XMA I

SEQ ID: 17 GGTACC ⇨KPN I

SEQ ID: 18 GAGCTC ⇨SAC I

SEQ ID: 19 GAATTC ⇨ECOR I

FIG. 10

VECTOR SIDE	OBJECT DNA FRAGMENT SIDE
HIND III	HINDIII
SPH I	SPH I
PST I	PST I
SAL I	SAL I
ACC I	ACC I
HINC II	HINC II
XBA I	XBA I
BAMH I	BAMH I
SMA I	SMA I
XMA I	XMA I
KPN I	KPN I
SAC I	SAC I
ECOR I	ECOR I
OTHER RESTRICTION ENZYME	
• • •	

FIG. 11

EP 0 095 358 B1

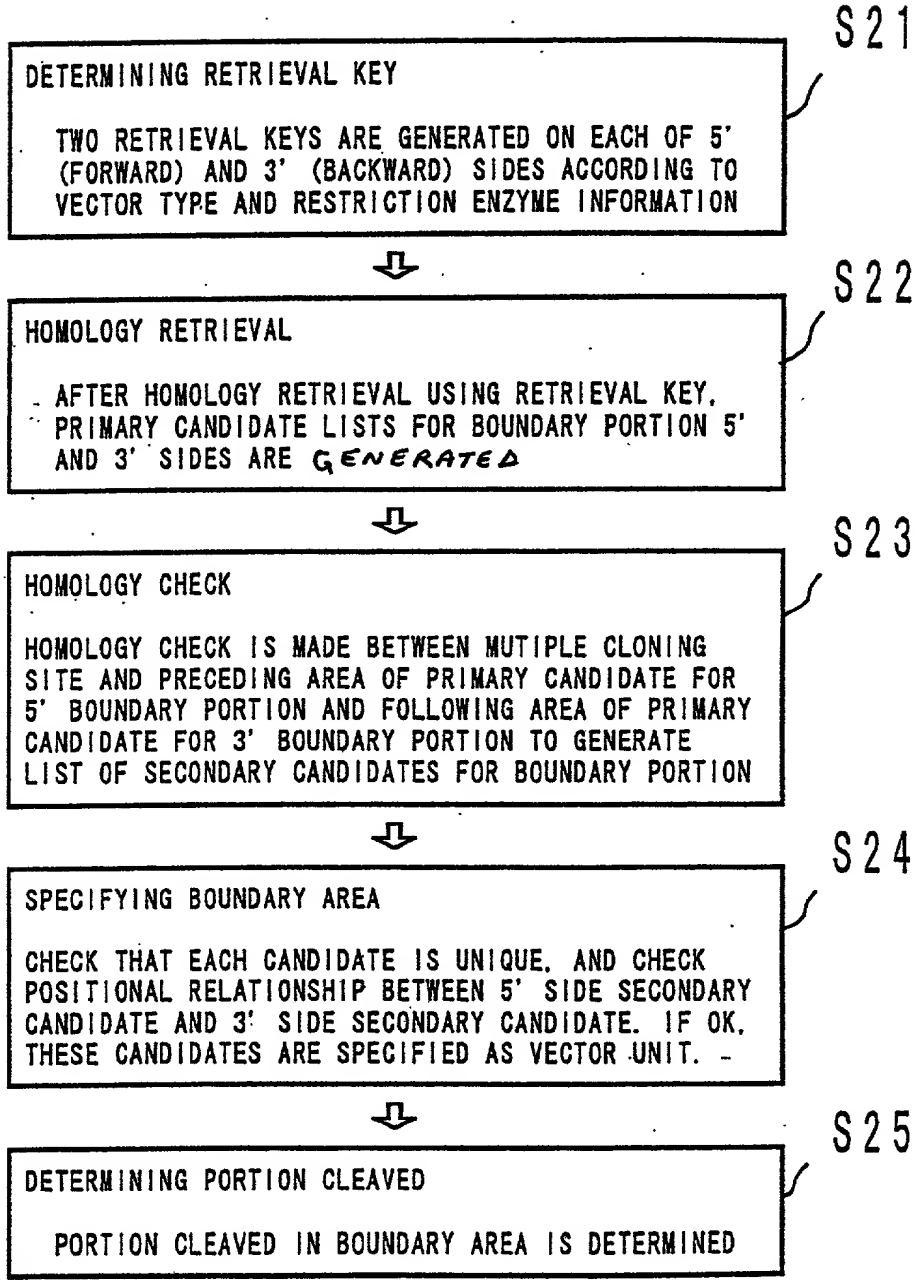

FIG. 12

EP 0 953 656 A2

WHEN SINGLE-STRANDED AREA IS FOUND ON 3' SIDE

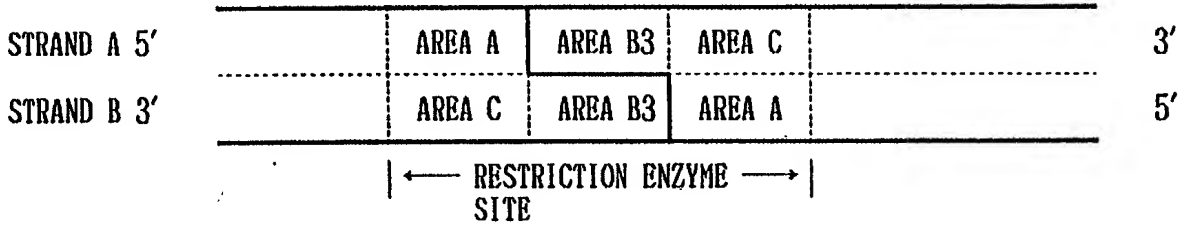

FIG. 13A

WHEN NO SINGLE-STRANDED AREA IS FOUND

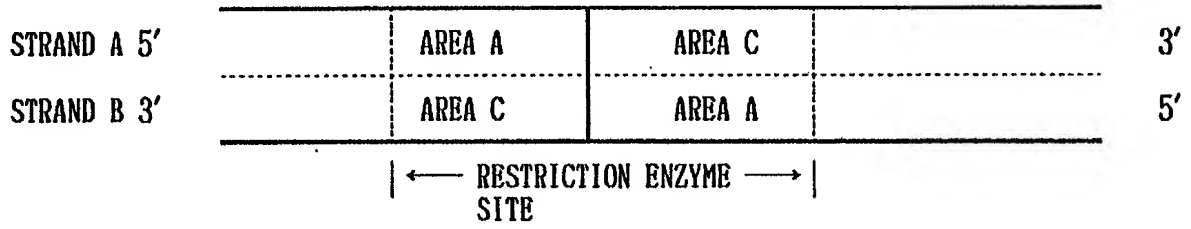

FIG. 13B

WHEN SINGLE-STRANDED AREA IS FOUND ON 5' SIDE

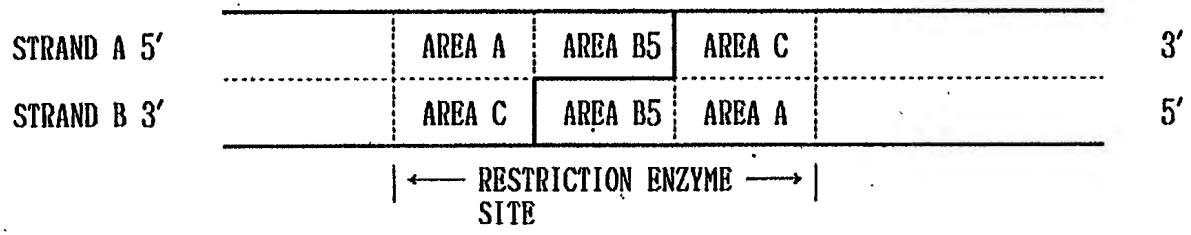

FIG. 13C

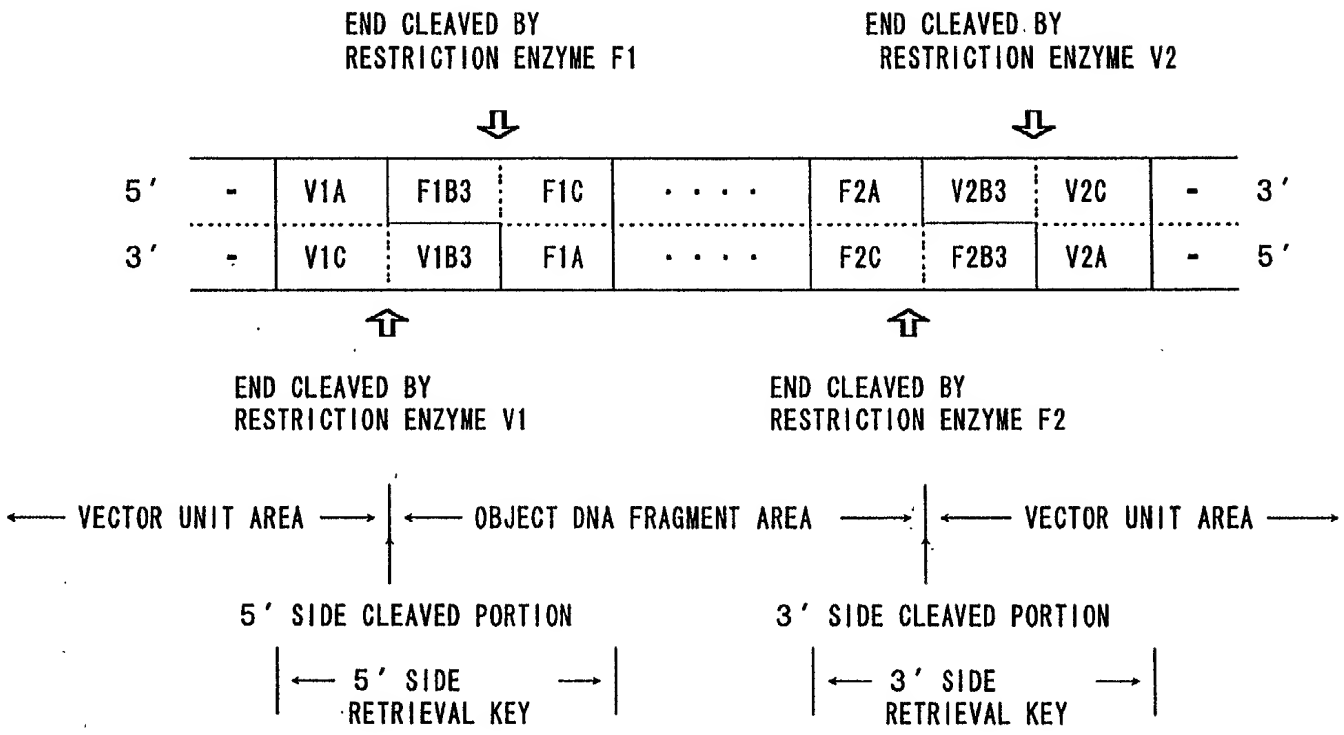
END CLEAVED BY
RESTRICTION ENZYME F1
END CLEAVED BY
RESTRICTION ENZYME V2
5′
3′
-
V1A
F1B3
F1C
· · · ·
F2A
V2B3
V2C
-
3′
-
V1C
V1B3
F1A
· · · ·
F2C
F2B3
V2A
-
5′
END CLEAVED BY
RESTRICTION ENZYME V1
END CLEAVED BY
RESTRICTION ENZYME F2
VECTOR UNIT AREA
OBJECT DNA FRAGMENT AREA
VECTOR UNIT AREA
5′ SIDE CLEAVED PORTION
3′ SIDE CLEAVED PORTION
5′ SIDE
RETRIEVAL KEY
3′ SIDE
RETRIEVAL KEY

FIG. 14

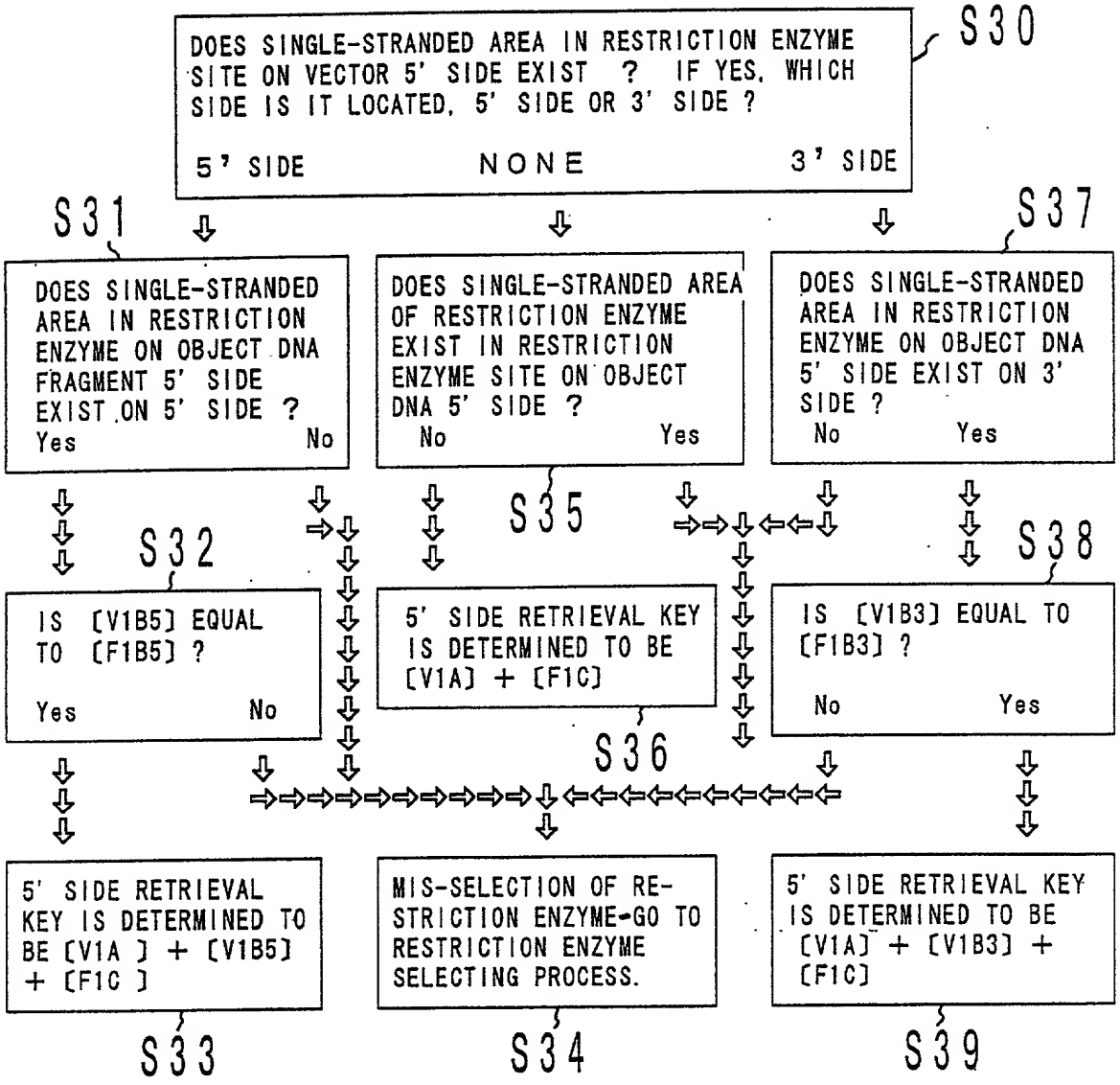
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME SITE ON VECTOR 5' SIDE EXIST ? IF YES, WHICH SIDE IS IT LOCATED, 5' SIDE OR 3' SIDE ?
5' SIDE
NONE
3' SIDE
S30
S31
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA FRAGMENT 5' SIDE EXIST ON 5' SIDE ?
Yes
No
S32
IS [V1B5] EQUAL TO [F1B5] ?
Yes
No
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [V1B5] + [F1C]
S33
DOES SINGLE-STRANDED AREA OF RESTRICTION ENZYME EXIST IN RESTRICTION ENZYME SITE ON OBJECT DNA 5' SIDE ?
No
Yes
S35
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [F1C]
S36
MIS-SELECTION OF RESTRICTION ENZYME-GO TO RESTRICTION ENZYME SELECTING PROCESS.
S34
S37
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA 5' SIDE EXIST ON 3' SIDE ?
No
Yes
S38
IS [V1B3] EQUAL TO [F1B3] ?
No
Yes
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [V1A] + [V1B3] + [F1C]
S39

FIG. 15

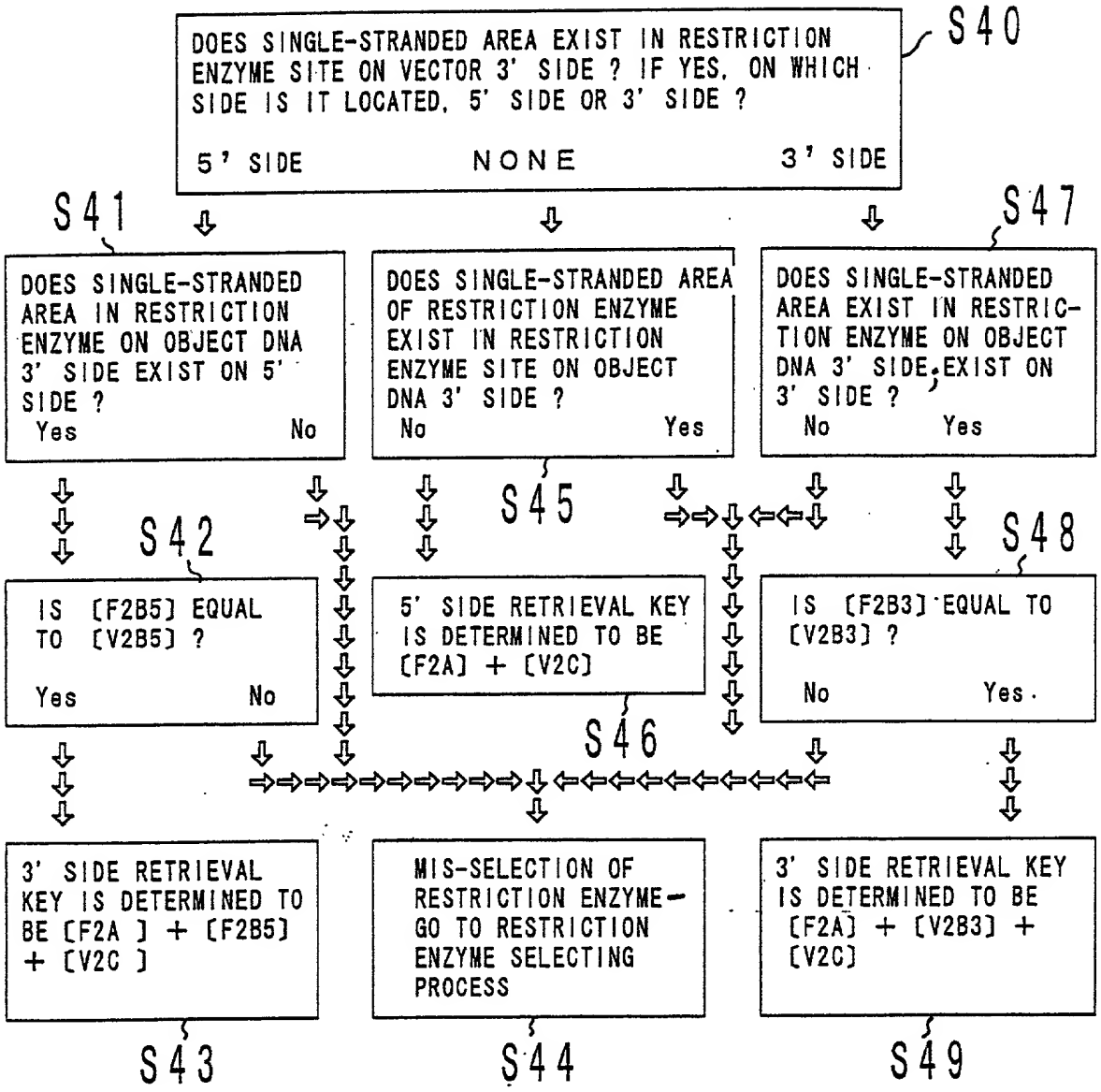
DOES SINGLE-STRANDED AREA EXIST IN RESTRICTION ENZYME SITE ON VECTOR 3' SIDE ? IF YES, ON WHICH SIDE IS IT LOCATED, 5' SIDE OR 3' SIDE ?
5' SIDE
NONE
3' SIDE
S40
S41
S47
DOES SINGLE-STRANDED AREA IN RESTRICTION ENZYME ON OBJECT DNA 3' SIDE EXIST ON 5' SIDE ?
Yes
No
DOES SINGLE-STRANDED AREA OF RESTRICTION ENZYME EXIST IN RESTRICTION ENZYME SITE ON OBJECT DNA 3' SIDE ?
No
Yes
DOES SINGLE-STRANDED AREA EXIST IN RESTRICTION ENZYME ON OBJECT DNA 3' SIDE, EXIST ON 3' SIDE ?
No
Yes
S42
S45
S48
IS [F2B5] EQUAL TO [V2B5] ?
Yes
No
5' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [V2C]
IS [F2B3] EQUAL TO [V2B3] ?
No
Yes
S46
3' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [F2B5] + [V2C]
MIS-SELECTION OF RESTRICTION ENZYME-GO TO RESTRICTION ENZYME SELECTING PROCESS
3' SIDE RETRIEVAL KEY IS DETERMINED TO BE [F2A] + [V2B3] + [V2C]
S43
S44
S49

FIG. 16

WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS SPECIFIED ON OBJECT DNA 3' SIDE

(**** INDICATES RESIDUAL MULTIPLECLONING SITE
(+++++ INDICATES AN OBJECT DNA FRAGMENT

```
    *****                          ********************************
GTGCCAAGCTT++++++++++++++++++TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT
    AAGCTT                     TCTAGA
      ⇧                          ⇧
```

5' SIDE RETRIEVAL KEY (IN THIS EXAMPLE, HIND III SITE)

9' SIDE RETRIEVAL KEY (IN THIS EXAMPLE, XBA I SITE)

FIG. 17

EP 0 454 438 A2

EP 0 956 520 A2

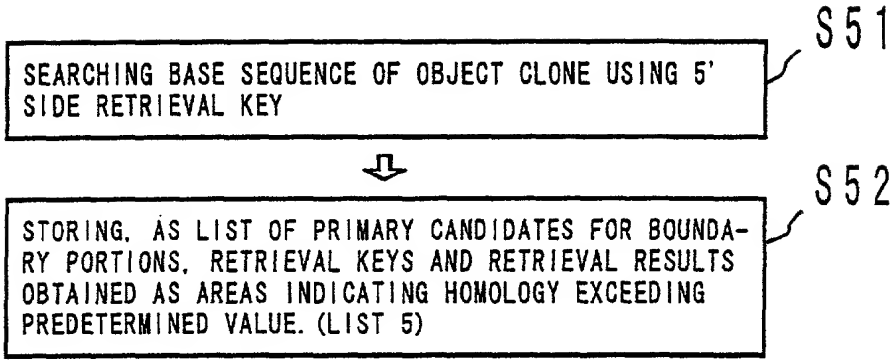

FIG. 18

EP 0 634 720 A1

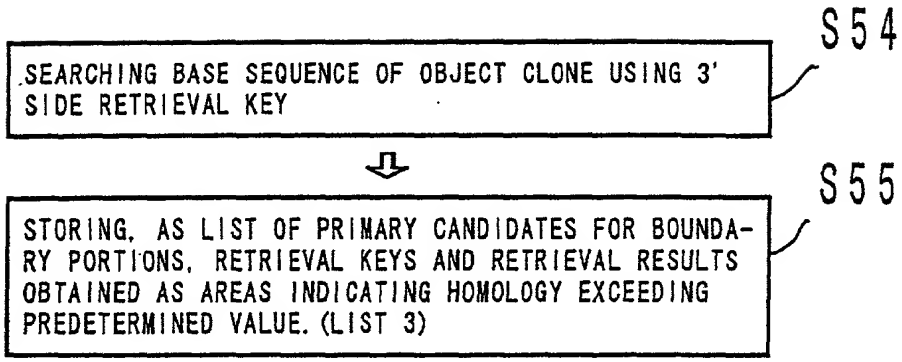

FIG. 19

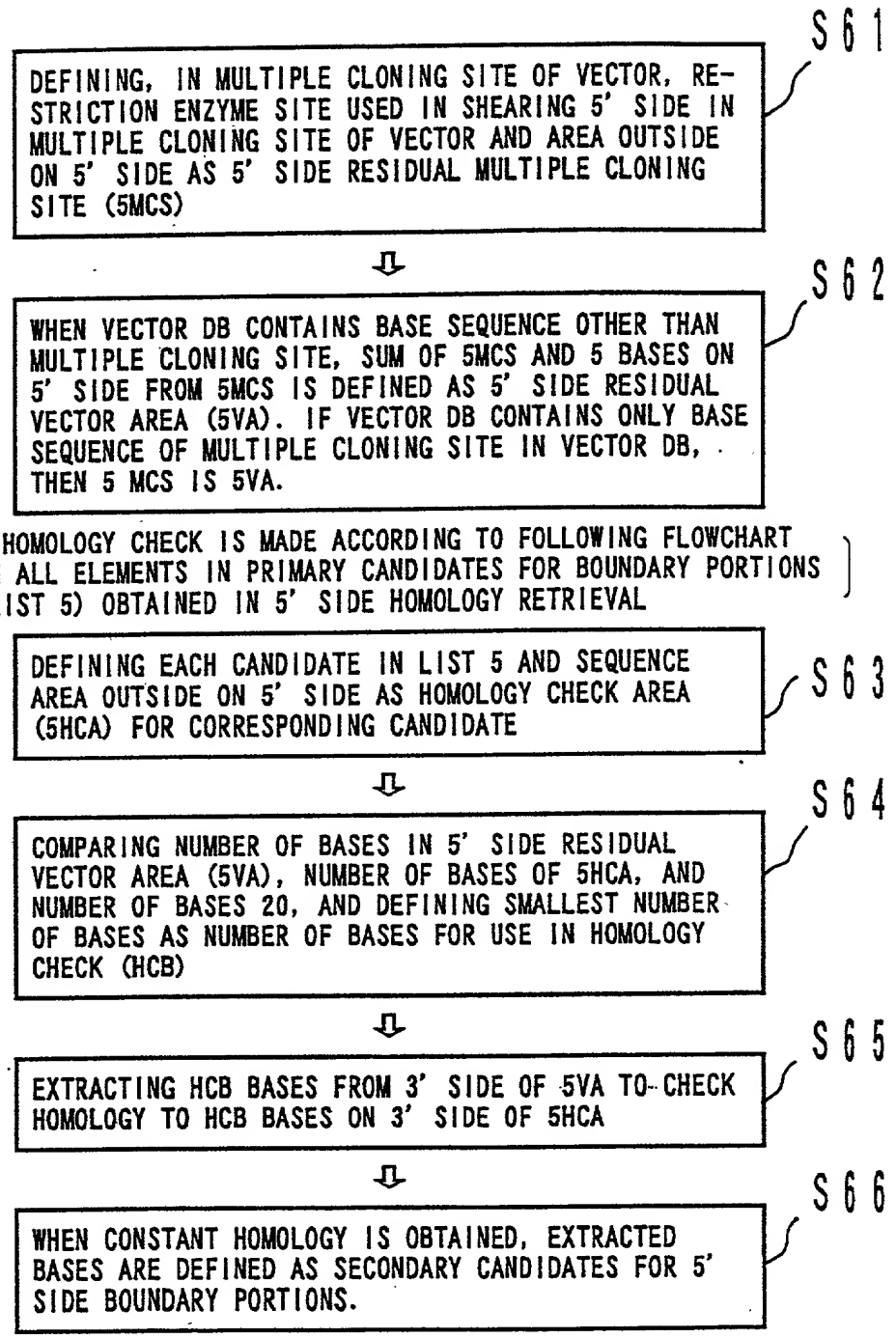

FIG. 20

EP 0 952 219 A1

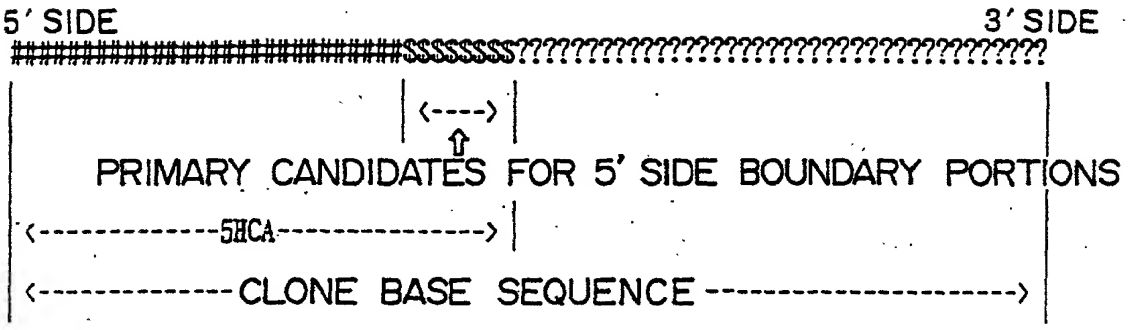

FIG. 21

0957529 - 052402

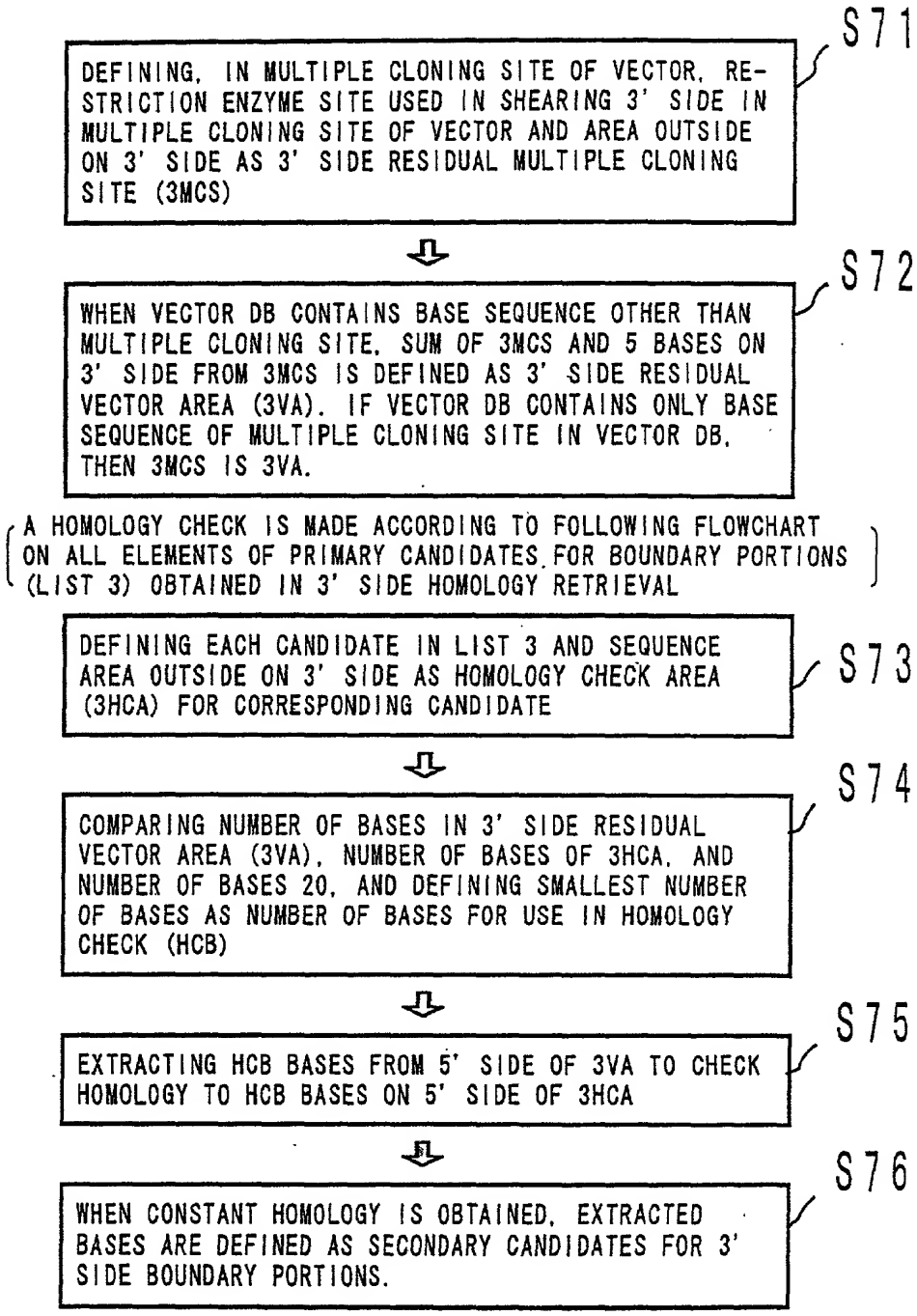

FIG. 22

EP 0 854 433 A2

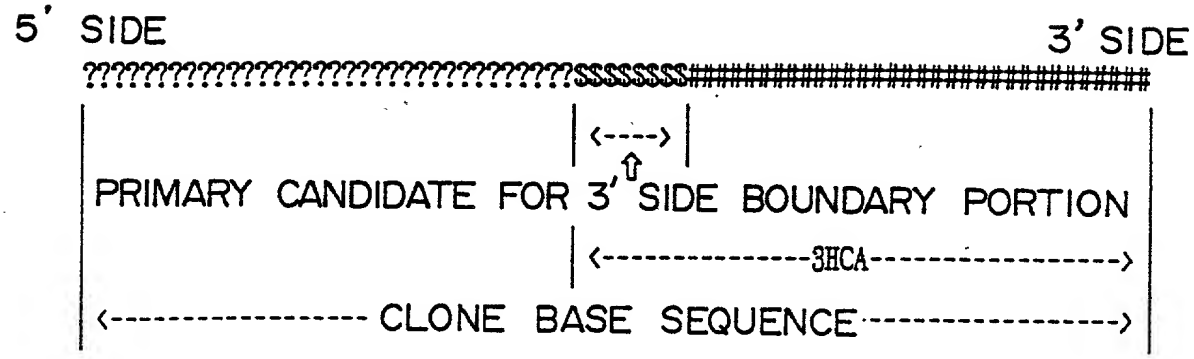

FIG. 23

Published - 05/25/89

FOR 2SD" 69259260

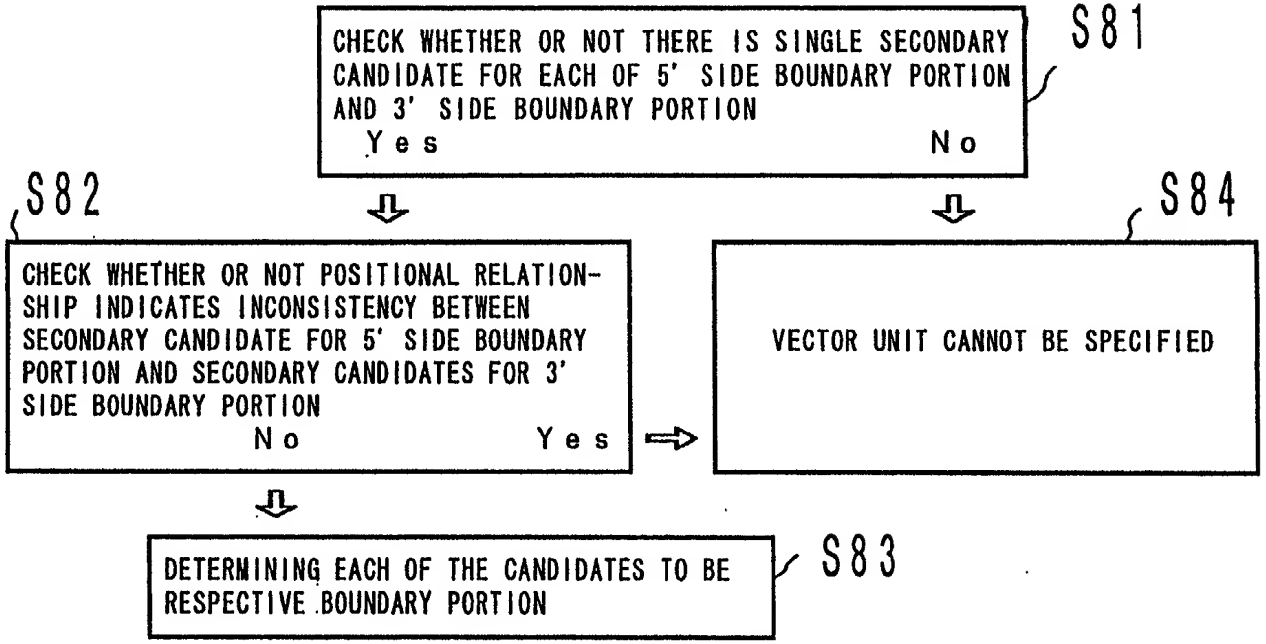

FIG. 24